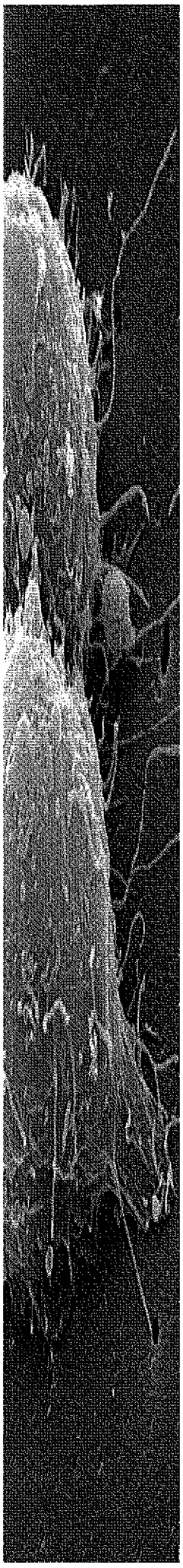

Tissue repair and stem cell renewal in carcinogenesis

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Cancer is increasingly being viewed as a stem cell disease, both in its propagation by a minority of cells with stem-cell-like properties and in its possible derivation from normal tissue stem cells. But stem cell activity is tightly controlled, raising the question of how normal regulation might be subverted in carcinogenesis. The long-known association between cancer and chronic tissue injury, and the more recently appreciated roles of Hedgehog and Wnt signalling pathways in tissue regeneration, stem cell renewal and cancer growth together suggest that carcinogenesis proceeds by misappropriating homeostatic mechanisms that govern tissue repair and stem cell self-renewal.

The tightly regulated growth of multicellular animals presents a striking contrast to single-celled organisms, which grow and divide in a manner limited only by nutrients available in the environment. The evolutionary compensation for loss of this exuberant style of growth comes in the form of organs that afford adaptability and efficiency through specialization for functions such as locomotion and reproduction, for sensing and responding to the environment, and for acquisition and use of nutrients. The assembly of such complex organs (pattern formation) requires mechanisms to establish intricate patterns of cell division and differentiation. Development of complex organs also takes longer than simple single-cell division, thus delaying the acquisition of reproductive maturity and exposing complex multicellular animals to a greater risk of tissue damage — whether from use, predation or exposure to a hostile environment.

The evolution of mechanisms that increase the complexity of animal form thus is likely to be coupled to the evolution of mechanisms for the renewal and repair of complex organs (pattern maintenance). Given this link, it is perhaps not surprising that pattern formation and pattern maintenance share common mechanisms, such as regulation by Hedgehog (Hh) and Wnt signalling pathways. These pathways play central roles in directing embryonic pattern formation, but also function post-embryonically in stem cell renewal, tissue repair and regeneration. Moreover, when aberrantly activated, these pathways can have important roles in the initiation and growth of cancer.

Here we focus on the relationship between the normal roles of Hh and Wnt pathways in pattern maintenance and on their pathological roles in the initiation and growth of malignant tumours. Using these pathways as central points of reference, we review recent developments in the area of cancer stem cells and the relationship of cancer stem cells to tissue stem cells; we concentrate in particular on stem cell renewal in the context of tissue repair as a common antecedent of cancer initiation.

Cancer, stem cells and cancer stem cells

A long-standing idea in cancer biology is that tumours arise and grow as a result of the formation of cancer stem cells, which may constitute only a minority of the cells within a tumour but are nevertheless critical for its propagation. The concept of cancer stem cells[1] dates back almost as far as the discovery of somatic stem cells in the haematopoietic system[2], and was firmly established experimentally in acute myelogenous leukaemia (AML)[3–5]. In these studies, a minority of undifferentiated cells isolated from leukaemic patients proved to be the only cells capable of reconstituting tumours on transfer into NOD/SCID (non-obese diabetic/severe combined immunodeficient) mice; the resulting tumours included a range of more differentiated cell types like those in the original leukaemia. In their cell-surface markers, in their multipotency and in their hierarchical self-renewal properties, these cancer stem cells resemble normal haematopoietic stem cells (HSCs), suggesting that the leukaemia stem cells either derive from HSCs or from more differentiated cells through acquisition of HSC properties.

Stem cells are appealing candidates as the 'cell of origin' for cancer because of their pre-existing capacity for self-renewal and unlimited replication[6,7]. In addition, stem cells are relatively long-lived in comparison to other cells within tissues. They therefore have more opportunity to accumulate the multiple additional mutations that may be required to increase the rate of cell proliferation and produce clinically significant cancers. The discovery of multipotent progenitor cells with the capacity for self-renewal (that is, stem cells) outside the haematopoietic system raises the possibility that cancer stem cells could arise from other tissue stem cells and initiate other cancer types, including solid cancers. Consistent with this possibility, a defined minority of cells within many human breast cancers are the only cells able to propagate the cancer in NOD/SCID mice, resulting in the reconstitution of tumours expressing the heterogeneous surface markers that were present in the original cancer[8]. In addition, cells with some of the properties of neural stem cells (NSCs), such as the ability to produce differentiated neurons and glia *in vitro* and *in vivo* and enhanced renewal activity, have recently been isolated from brain tumours and brain-tumour-derived cell lines[9–11].

The general validity of the cancer stem cell concept has not been proven, as the number of cancer types for which cancer stem cells have been identified is limited. This is, however, an important issue as successful therapy depends on targeting the cells within a tumour that drive cancer growth. However, as many cancers are heterogeneous both in their cell composition and in the relative abundance of cells capable of propagating tumour growth, it will not be surprising if cancer propagation turns out to be an exclusive property of defined subsets of cells within many particular cancer types. The key to developing effective future therapies thus seems to be the identification and characterization of these cancer stem cells, and the development of drugs that specifically target them.

Another important issue for understanding the origins of cancer is the relationship between cancer stem cells and normal tissue stem cells. The best-studied cancer stem cells are those in AML, which have been isolated and individually marked before being serially transferred through several host animals[3–5]. In most respects examined so far, these cells resemble normal HSCs, consistent with a stem cell origin for the AML-cancer stem cell. The possibility cannot be excluded, however, that cancer stem cells might be derived from more committed progenitors by genetic or epigenetic changes that confer self-renewal ability[12]. Genetic or epigenetic changes that bestow and activate the ability to self-renew on a committed progenitor cell seem less likely to occur than changes that activate renewal in a stem cell — particularly as the window of cellular plasticity within which committed progenitors might acquire renewal ability is generally limited by progression towards irreversible differentiation and replicative quiescence. De-differentiation of committed progenitors back to stem cells under tightly controlled genetic conditions has nevertheless been reported in the *Drosophila* testis and ovary. For example, prematurely differentiated spermatogonia in the *Drosophila* testis can be induced to regenerate male germline stem cells on restoration of a critical signalling pathway, Jak–STAT (Janus kinase–signal transducers and activators of transcription)[13]. Moreover, cystocytes that have already begun to form oocytes within the ovary can be induced to de-differentiate to form productive female germline stem cells on ectopic activation of signalling by Dpp (Decapentaplegic), the *Drosophila* homologue of BMP4 (bone morphogenetic protein 4)[14], a member of the TGF-β (transforming growth Factor-β) family.

Of particular recent interest in the origin of cancer is the observation that transient Hh and Wnt pathway activities promote stem cell self-renewal in normal tissues, whereas continuous activation is associated with the initiation and growth of many types of human cancer. These pathways thus provide a potential link between the normal self-renewal of stem cells and the aberrantly regulated proliferation of cancer stem cells.

Hh and Wnt signalling in stem cell maintenance

In addition to their well-established roles in directing the patterning of embryonic tissues and structures (reviewed in refs 15–17), the Hh and Wnt pathways have more recently been implicated in the maintenance of stem or progenitor cells in a growing list of adult tissues that now include skin, blood, gut, prostate, muscle and the nervous system[18–29] (Table 1). Evidence for a role of these pathways in stem cell maintenance functions comes from genetic interventions *in vivo* or the treatment of isolated stem cells *in vitro* (in the case of HSCs and NSCs), followed by assays for proliferation, engraftment and multilineage potential of presumptive stem cell populations. A general feature of the results of these studies is that Wnt and Hh pathway activities seem to increase presumptive stem cell number by stimulating stem cell proliferation. Thus, for example, loss of Hh signalling does not immediately obliterate hippocampal populations of neural stem cells, but affects their number by decreasing their proliferative capacity, both *in vivo* and *in vitro*[19,20]. A similar effect of Hh pathway activity on numbers of somatic stem cells (follicle stem cells) has been noted in the *Drosophila* ovary[18]. Likewise, *in vitro* treatment of isolated HSCs with Wnt or Hh proteins increases their proliferative capacity and improves their ability to form colonies *in vitro* and to colonize NOD/SCID mice[22,27]. Similarly, loss or inhibition of Wnt pathway activity in the intestine does not abrogate the initial development of normal epithelial architecture, but instead causes a progressive degradation of epithelial structure. This effect is associated with the loss of proliferative activity in the crypts, where stem cells reside[21,26].

In contrast to these effects of Hh and Wnt pathway activities on stem cell self-renewal, other signals within the stem cell niche seem to function more immediately in the maintenance of stem cell identity. For example, the Jak/STAT and TGF-β signalling pathways seem to specify male and female germ cell identity in the *Drosophila* testis and ovary, respectively; genetic manipulation of these pathways rapidly and qualitatively alters cell phenotypes[13,14]. Signals for stem cell maintenance might therefore be classified as signals with immediate effects on the maintenance of stem cell identity, and signals that regulate renewal divisions. Identity maintenance functions cannot be ruled out for Hh and Wnt pathway activities, but most evidence points to stem cell renewal as the main target in most tissues. A role of these pathways in normal stem cell renewal is consistent with their known role in the regulation of stem cell renewal genes such as *nestin* (encoding an intermediate filament protein) and *Bmi-1* (encoding a component of the Polycomb transcriptional-silencing complexes) in tumours that depend on Hh or Wnt signalling (discussed in section 'Cancer and persistent states of repair' below).

Hh and Wnt signalling in cancer

The roles of Hh and Wnt pathways in stem cell renewal are particularly interesting given the genetically implied connection between activity of these pathways and the initiation and growth of a substantial fraction of lethal cancers (Table 2). Familial mutations that facilitate Hh and Wnt pathway activation have been associated with increased incidence of specific brain, skin, skeletal muscle, liver and colon cancers in humans and mice, and of bladder cancer in mice. Additional studies in which pathway activities are antagonized by treatment with pharmacological agents, with antibodies that bind and block ligand action, or by overexpression of negatively acting pathway components further demonstrate an ongoing requirement for pathway activity in the growth of additional cancer types which include small-cell lung cancer and carcinomas of the oesophagus, stomach, pancreas, biliary tract and prostate. The range of organs from which Hh- and Wnt-pathway-dependent cancers originate is therefore similar to the range of organs in which these pathways have a role in stem cell renewal. In terms of medical significance, about one-third of total cancer deaths are caused by the cancer types in which current evidence implicates Hh or Wnt pathway activity in most cases[30].

The Hh pathway in cancer

The link between Hh pathway activity and cancer was initially established by the identification of heterozygous mutations affecting Patched (PTCH), a negatively acting component of the Hh receptor, as the cause of Gorlin's syndrome. This syndrome is associated with an increased incidence of basal cell carcinoma, medulloblastoma, and rhabdomyosarcoma, and PTCH is also mutated in sporadic forms of

Table 1 **Patterning pathways and stem cell maintenance**

Pathway	Tissue	Evidence	References
Hh	NSC	Hh required for NSC proliferation in neurospheres; conditional inactivation of SMO inhibits NSC proliferation *in vitro* and *in vivo*	19, 20
	HSC	*In vitro* and *in vivo* expansion of HSCs by Shh	27
	Drosophila ovary	Proliferation of ovarian somatic stem cells requires Hh	18
Wnt	HSC	Decreased proliferation and colony-forming ability of HSCs subjected to Wnt pathway inhibition	22
		Increased number of haematopoietic progenitors from mouse fetal livers and human bone-marrow cells stimulated with Wnt ligand	84, 85
	Intestine	TCF4-null mice have diminished intestinal stem cell pool	21, 26 and refs therein
	Muscle	Culture with Wnt ligand induces myogenic stem cells; Wnt antagonism with secreted Fz proteins reduces muscle stem cell proliferation	23
	Mammary gland	Increased progenitors in transgenic mice with activated Wnt signalling	65

TCF4, a TCF family member (see text; Fig. 1).

these cancers, thus identifying PTCH as a tumour suppressor (reviewed in refs 6, 31). A similar range of tumours was also found to be associated with sporadic activating mutations affecting the positive receptor component and proto-oncogene Smoothened (SMO). In normal Hh pathway function, the transporter-like Ptch protein acts catalytically to restrain activation of the seven-transmembrane protein Smo. Ptch activity is blocked by binding of Hh, which liberates Smo for activation of transcriptional targets through the Gli family of latent transcriptional factors (see Fig. 1a). These features of Hh signalling are broadly conserved between *Drosophila* and mammals, as are common mechanisms for Hh protein processing and lipid modification and a dedicated mechanism for the release of lipid-modified Hh protein from producing cells. (reviewed in refs 32, 33). In addition the Gli proteins, like their *Drosophila* counterpart Ci, can be regulated by interactions with the Suppressor of fused (Su(fu)) protein and can exist in activating forms (primarily Gli and Gli2) as well as in proteolytically processed repressing forms (primarily Gli3). The human SU(FU) gene has also been implicated as a tumour suppressor, with mutations found in familial and sporadic medulloblastoma and in sporadic basal cell carcinoma (see Table 2).

Despite extensive similarities between *Drosophila* and mammalian pathways, however, significant differences may exist, particularly in the transductory machinery between Smo and Gli. Thus, although recent genetic and biochemical studies in *Drosophila* have demonstrated that pathway activation is transmitted through association of Smo with a complex of cytoplasmic proteins that includes Ci and a kinesin-like protein, Cos2, a functional mammalian homologue of Cos2 has not been identified (reviewed in ref. 32). Because of its role in maintenance of pathway quiescence in *Drosophila*, a functional mammalian Cos2 homologue would be of interest as a potential tumour suppressor. In addition, several apparent pathway components identified in mammals either have no counterparts or do not function in the *Drosophila* Hh pathway (see ref. 34). These include components such as RAB23 (ref. 35) or FKBP8 (ref. 34), which have unknown function, but are of interest as potential tumour suppressors because of their action downstream of Smo as negative regulators of pathway activity (see Fig. 1).

Some tumours of the type associated with Gorlin's syndrome are not associated with known pathway-activating mutations, despite clear evidence for pathway activity[36,37]. This suggests that activation of the Hh pathway may occur through mechanisms other than by mutation of pathway components, and raises the possibility that such mechanisms may also have a role in pathway activation in other cancers not typically associated with Gorlin's syndrome. Consistent with this possibility, recent studies using cyclopamine, a specific Hh pathway antagonist[38–40], indeed have demonstrated an ongoing requirement for pathway activity in the growth of a series of lethal cancers arising in organs of endodermal origin, and not typically associated with Gorlin's syndrome. These cancers include small-cell lung cancer and carcinomas of the oesophagus, stomach, pancreas, biliary tract, and prostate[41–43]. Pathway activity in these cancers requires ligand activation, as demonstrated with the use of Hh-blocking antibodies, and contrary to ligand-independent activation arising in tumours associated with Gorlin's syndrome. Curiously, the limiting factor in pathway activation in these non-Gorlin's tumours seems not to be ligand expression, but rather the acquisition of responsiveness to ligand. Thus, whereas the Hh family members Shh (Sonic hedgehog) and Ihh (Indian hedgehog) are expressed in normal endodermal tissues, high-level activation of Hh pathway targets occurs only in cancer cells. In the prostate, the limiting factor for ligand responsiveness is SMO, which is not expressed in normal prostate tissue[29]. Furthermore, isolated prostate stem/progenitor cells acquire Hh responsiveness simply by introduction of Smo expression constructs, and these cells are oncogenically transformed upon pathway activation. The genetic or epigenetic changes that trigger Smo expression are not identified, although they may be linked to epithelial regeneration (see section 'Cancer and persistent states of repair' below).

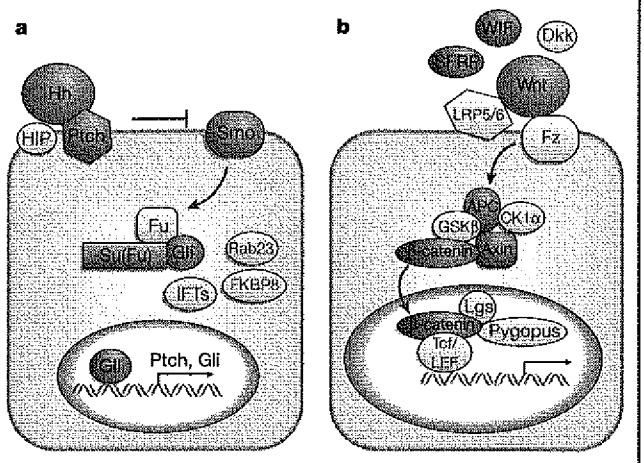

Figure 1 Hh and Wnt signalling pathways. Simplified views of the Hh and Wnt signalling pathways, with emphasis on components implicated in cancer or tissue regeneration. Green and red colours denote pathway components with primarily positive or negative roles, respectively, in pathway activation. Shaded components have been causally implicated in tumorigenesis (see Table 2 and text; more complete pathway descriptions are available in refs 32–34 for Hh and refs 17, 46 for Wnt). **a**, Activation of the Hh signalling pathway is initiated by binding of a Hh ligand to Ptch. This lifts suppression of Smo, activating a cascade that leads to the nuclear translocation of Gli and the activation of target genes. HIP is a membrane protein that antagonizes pathway activity by binding to Hh ligands, and Fu, Su(Fu), Rab23, FKBP8 and the IFTs (intraflagellar transport proteins) act downstream of Ptch and Smo to regulate Gli. The function of Rab23, FKBP8 and the IFTs outside the CNS is not established. HIP, Hh-interacting protein; Rab23, a member of the Rab family of GTPases; FKBP8, a member of the FK506-binding protein family. **b**, The Wnt signalling pathway is activated by binding of Wnt ligands to their receptors Fz and LRP5/6, leading to the release of β-catenin from the degradation complex and facilitating its entry into the nucleus, where it regulates target gene transcription through association with Tcf/LEF. Legless (Lgs) and Pygopus. SFRP, WIF and Dkk are secreted antagonists of Wnt signalling. APC, Axin, GSK3β and CK1a are components of the β-catenin degradation complex. WIF, Wnt inhibitory factor; Dkk, Dickkopf; GSK3β, glycogen synthase kinase 3β; CK1a, casein kinase 1a.

The Wnt pathway in cancer

The Wnt signalling pathway has also been implicated in several types of cancer, initially through overexpression of the Wnt-1 protein signal in murine mammary tumours as a consequence of nearby mouse mammary tumour virus (MMTV) insertion[44], and subsequently through the substantially increased incidence of colorectal and other cancers in familial adenomatous polyposis, caused by mutations affecting the tumour suppressor *APC* (adenomatous polyposis coli) (reviewed in ref. 45). In the absence of Wnt signal, APC fosters the degradation of the oncogene β-catenin and prevents its entry into the nucleus (Fig. 1b). Wnt stimulation, loss of APC protein function, or of its associated partner Axin, all lead to the stabilization of β-catenin and to its increased concentration in the nucleus. β-catenin can then act as a transcriptional co-activator by associating with the Tcf/LEF family of transcription factors. A complex of APC with Axin and other proteins targets β-catenin for proteasomal degradation by scaffolding an association between β-catenin and kinases whose activities lead to β-catenin ubiquitinylation. This action is abrogated by the recruitment of the degradation complex to the membrane upon Wnt activation of a receptor complex that includes Frizzled (Fz), a relative of Smo, and LRP5/6. Any lesion causing β-catenin accumulation through the disruption of a degradation complex component or by mimicking complex recruitment to the receptor would be expected to promote tumour formation. This pathway can also be activated by mutations of β-catenin that render it resistant to degradation (for detailed reviews of Wnt signalling see refs 6, 17, 46).

Table 2 **Hh and Wnt pathways in cancer**

Tissue	Tumour	Evidence of pathway involvement	References
Hh pathway			
Brain	Medulloblastoma	Tumorigenesis by inactivation of *PTCH*; allograft and cell-line growth inhibition by cyclopamine; inhibition of autochthonous tumour growth by synthetic small molecule antagonist	37, 81; reviewed in 6
		Tumorigenesis by inactivation of Su(fu)	86
	Glioma	Gli amplification; growth inhibition of some cell lines by cyclopamine	87, 88
Skin	Basal cell carcinoma	Tumorigenesis by inactivation of *PTCH*; *in vivo* tumorigenesis by expression of activating form of *SMO* or by Shh overexpression and *in vitro* growth inhibition by synthetic Hh pathway antagonist; inhibition of human tumour growth topical cyclopamine	82, 83; reviewed in 6
Muscle	Rhabdomyosarcoma	Tumorigenesis by inactivation of *PTCH*	reviewed in 6
Oesophagus	Adenocarcinoma	Cell-line growth inhibition by cyclopamine, Hh blocking antibody	42
Stomach	Adenocarcinoma	Cell-line growth inhibition by cyclopamine, Hh blocking antibody	42
Pancreas	Adenocarcinoma	Xenograft and cell-line growth inhibition by cyclopamine, Hh blocking antibody; tumour initiation (in mouse) by Shh overexpression	42, 43
Biliary tract	Adenocarcinoma	Xenograft and cell-line growth inhibition by cyclopamine, Hh blocking antibody	42
Lung	Small-cell lung cancer	Xenograft and cell-line growth inhibition by cyclopamine, Hh blocking antibody	41
Prostate	Adenocarcinoma	Xenograft and cell-line growth inhibition and suppression of metastasis by cyclopamine; increased xenograft growth by Shh and Gli overexpression	29, 89, 90
Bladder	Urothelial carcinoma	Increased tumour induction (in mouse) by alkylating agent in *Ptch* heterozygote	91
Oral cavity	Squamous cell cancer	Growth inhibition of cell lines by cyclopamine;	92
Wnt pathway			
Colon	Adenocarcinoma	Tumorigenesis by inactivation of APC, Axin; tumorigenesis by stabilization of β-catenin; epigenetic inactivation of SFRP's	47; reviewed in 45
Liver	Hepatoblastoma	Tumorigenesis (in mouse) by inactivation of APC and by stabilization of β-catenin	reviewed in 45
Blood	Multiple myeloma	Cell growth inhibition by dominant negative TCF4; growth stimulation by Wnt ligand	93
Hair follicle	Pilomatricoma	Tumorigenesis (in mouse) by overexpression of β-catenin	reviewed in 45
Bone	Osteosarcoma	Dkk3 and LRP5 expression inhibits tumour cell growth *in vitro*	94, 95
Lung	Non-small-cell carcinoma	Apoptosis and cell-growth inhibition by short interfering RNA and a blocking antibody against Wnt2	96
Pleura	Mesothelioma	Apoptosis and cell-growth inhibition by transfection of SFRP	97

Emphasis is placed on functional data showing a requirement for pathway activation in tumour formation and/or tumour cell growth. (See Fig. 1 and text for gene abbreviations.)

Hh and Wnt signalling pathways are similar in that both signals are lipidated (an important process that affects their activity and tissue distribution[33]), and they use several related or identical components. The fundamental logic of pathway activation is also similar, in both cases involving receptor recruitment of multicomponent complexes with key roles in cytoplasmic anchoring and proteolysis of key transcriptional effectors. It is also possible that, like Hh, the Wnt pathway is activated in a wider range of cancers than has been revealed by familial or sporadic mutations that produce ligand-independent pathway activation. The possibility of Wnt ligand dependent pathway activation in cancer is suggested by a recent study demonstrating that epigenetic silencing of *SFRPs* (secreted frizzled-related proteins), which encode an extracellular ligand-binding pathway antagonist, may have a critical role in the early establishment of colorectal cancer[47]. A broader range of cancers requiring Wnt pathway activity for growth may also be revealed, as potent and specific Wnt pathway antagonists are identified and become broadly available[48].

Hh and Wnt pathways in regeneration and tissue repair

If cancer stem cells arise from tissue stem cells, and if Hh and Wnt pathway activities are critical for the renewal of at least some of these stem cell types, then continuous Hh and Wnt pathway activities may promote cancer growth by continuously recapitulating their roles in promoting normal stem cell renewal. But stem cell renewal must be tightly regulated (otherwise tumours might arise), raising the critical question of how and under what circumstances normal regulation can be circumvented in cancer.

Some insight into the regulation of stem cell renewal activity may be gained from a consideration of the role of Hh and Wnt pathways in regenerative responses (Table 3). Wnt pathway activation in the radially symmetric coelenterate *Hydra* is closely associated with the growth and patterning of new individuals. This may result either from normal asexual budding or from experimental manipulations, such as cell dissociation and re-aggregation[49]. *Hydra* tissue thus seems to exist in a constant state of growth and replacement. Amphibia, particularly urodeles (newts and salamanders), are also capable of mounting impressive regenerative responses to limb amputation or to extirpation of certain organs. The typical sequence of events involves de-differentiation of cells near the site of injury, followed by extensive proliferation and patterning of the regenerating tissues. In the cases of urodele limb and lens regeneration, Hh family members are expressed in the de-differentiated cells following injury, and regeneration can be blocked by treatment with cyclopamine[50–52]. Fin regeneration in fish also entails expression of Hh genes and targets, and is disrupted by cyclopamine inhibition[53,54].

The regenerating structures in these examples encompass several tissue types that are arranged in complex patterns; in this respect pathway roles resemble those in embryonic pattern formation. But Hh and Wnt pathway activity also have a role in regenerative responses that are restricted to single tissue types within organs. For example, transient Hh pathway activity is required for androgen-triggered regeneration of prostate epithelium in male mouse castrates[29], and Wnt pathway activities similarly are required for muscle regeneration in response to cardiotoxin-induced muscle injury[23]. Increased Hh pathway activity in $Ptch^{+/-}$ mice also contributes to an increase in photoreceptor-cell progenitor number and retinal repair in a model of retinal degeneration. Furthermore, mammary progenitors are enriched in mice with Wnt pathway activation caused by increased Wnt ligand levels or by a β-catenin altered to increase its stability[55]. In addition to these demonstrations of functional Hh and Wnt pathway activity in tissue repair, correlative data suggest a possible role for Wnt pathway activity in response to biliary tract[56], liver[57] and kidney tubule injury[58], and for Hh pathway activity in repair of bone fractures[59], bile duct[56] and airway injury[41].

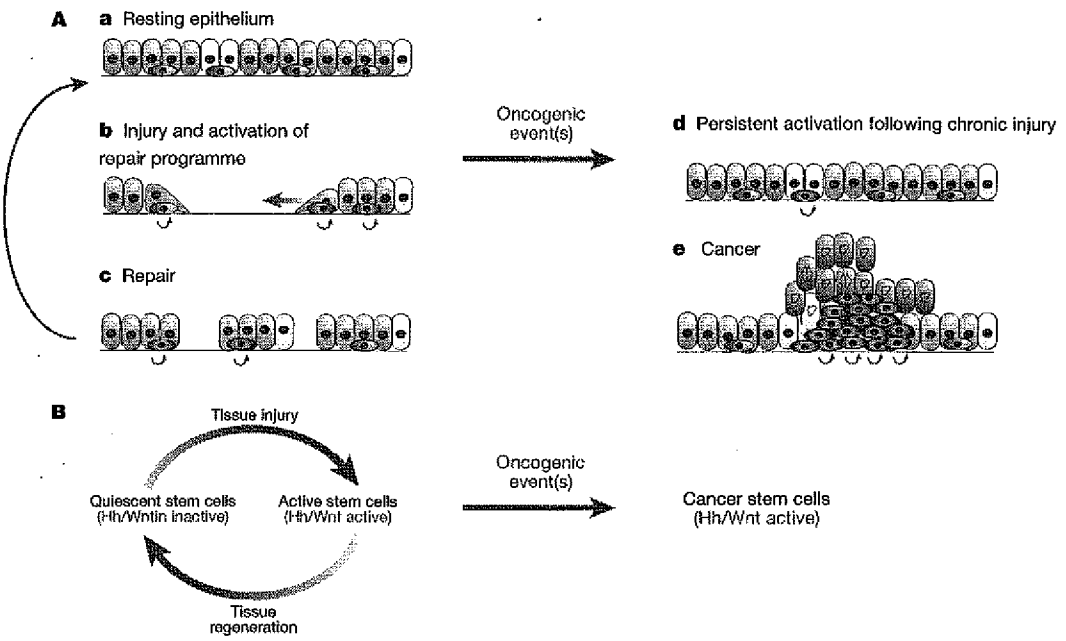

Figure 2 Model for carcinogenesis resulting from persistence of a state of injury repair. **A**, Cellular events of epithelial repair. **a**, Resting epithelium with several differentiated cell phenotypes (brown, orange, and yellow) derived from tissue stem cells, now quiescent (red). Pathways such as Hh and Wnt signalling pathways that have a role in the renewal of stem cells are not active. **b**, Epithelial defect resulting from acute injury. Loss of epithelial continuity activates a repair program which is driven by Hh or Wnt signalling. This program results in the acquisition by epithelial cells of a more mesenchymal phenotype, including flattening and movement of cells (straight arrow) to cover the wound, activation (green), and expansion of stem cells through renewal divisions (curved arrows). **c**, The wound is repaired, first by rapid cell movement, and then by restoration of cell numbers resulting from the amplification of stem cells and the differentiation of their progeny. Subsequently, either epithelial continuity and patterning is restored, Hh and Wnt signalling ceases, and the stem cell compartment returns to quiescence (**a**), or oncogenic event(s) may trap a stem cell in an activated state of continuous renewal, which is driven by autonomous Wnt or Hh signalling (**d**). Further genetic or epigenetic change in such a persistently activated stem cell (curved red arrows) might produce a cancer stem cell (green) which is capable of aggressively propagating a cancer (**e**). This may result from enhanced proliferation and production of more cancer stem cells as well as from differentiated cancer cells (blue). **B**, Stem cells cycle between quiescence and activity as a consequence of Hh/Wnt driven responses to injury. Oncogenic event(s) may trap activated stem cells in a permanent state of Hh/Wnt driven activity, resulting in cancer stem cells.

Cancer and persistent states of repair

We have reviewed evidence highlighting the role of Hh and Wnt pathway activity in cancer growth on the one hand, and in stem cell renewal and tissue regeneration on the other. But is there a link between tissue repair and cancer? A connection is strongly suggested by the known association between chronic tissue injury and cancer[60,61], including cancers associated with Hh and/or Wnt pathway activity. Increased cancer risks are associated with exposure to toxins, such as alcohol, cigarette smoke and organic chemicals[62–64], with chronic infection of *Helicobacter pylori* and other pathogens[65], and with inflammatory conditions, such as sclerosing cholangitis and inflammatory bowel disease[66,67] — all of which entail chronic tissue damage. As discussed above, acute injury is accompanied by the expansion of stem cell pools and by the transient activation of the Hh and Wnt signalling pathways[41]. Under conditions of chronic injury, pathway activation and presumed expansion of stem cell pools would persist so long as repeated injury prevents full regeneration. This state of continuous pathway and progenitor-cell activation resembles the continuous pathway activity and cell division seen in cancer.

These observations suggest that cancer growth may represent the continuous operation of an unregulated state of tissue repair and that continuous Hh/Wnt pathway activities in carcinogenesis may represent a deviation from the return to quiescence that normally follows regeneration (Fig. 2A, a, b). The simplest model for the emergence of this state is that genetic or epigenetic events prevent the return to quiescence of an activated stem or progenitor cell on completion of regeneration, thus initiating a tumour by trapping the cell in an activated state of continuous renewal. Consistent with this model, the *Bmi-1* gene required for HSC renewal is also required for the propagation of leukaemias in transfer experiments[68,69]. The expression of Bmi-1 and nestin, which are both associated with stem cell renewal[68–70], is dependent on Hh pathway activity in Hh-dependent tumours[29,37,41,71]. Of course, multiple genetic or epigenetic changes might be required to trap the activated stem cell initially, and numerous other events may contribute to rapid proliferation or to other aspects of the phenotype. Conversion of an activated stem cell into a clinically threatening cancer stem cell may involve changes that lock the cell in an active state of renewal and allow the cell to acquire independence from niche signals that are normally required to maintain stem cell identity.

The observed increase in cancer incidence associated with chronic injury strongly supports this model of cancer as a continuous state of repair. If, as hypothesized, the oncogenic event results in trapping activated stem cells in a continual state of renewal, then any condition that increases the pool size of activated stem cells should increase the probability of an oncogenic event by making the cellular substrates for such an event more numerous. The effect of repeated injury over time would be exactly this — to increase the pool size of stem cells in an active state of renewal (Fig. 3). Tissues that normally undergo rapid renewal might also be expected to experience increased cancer incidence, as a high turnover rate might require a sizeable pool of activated stem cells. Indeed, organs such as the skin, the lungs, and the gastrointestinal tract, which are continuously exposed to environmental insults, and consequently in a constant state of renewal, are the tissues of origin for a high proportion of cancers.

Table 3 **Hedgehog and Wnt signalling in regeneration**

Organism	Organ/tissue	Injury	Components induced during regeneration	Pathway modulation and effect	References
Hh pathway					
Newt	Lens	Lensectomy	Shh, Ihh, Ptch	Regeneration blocked by *KAAD-cyclopamine and HIP-transfection	50
	Limb	Amputation	Shh, Ptch	Regeneration blocked by cyclopamine	51, 52
Zebrafish	Fin	Amputation	Shh, Ptch, Bmp2	Regeneration blocked by cyclopamine	53, 54
Mouse	Vasculature	Ischaemia	Shh, Dhh, Ptch	Regeneration blocked by anti-Hh antibody	98
	Prostate	Androgen deprivation by castration	Ptch	Regeneration blocked by cyclopamine and anti-Hh antibody	29
	Retina	Degeneration	Ptch	Mice with Ptch mutations show improved retinal photoreceptor regeneration	99
	Facial nerve	Axotomy	Shh, Smo	Regeneration reduced by cyclopamine	100
	Bile duct	Immune	Ptch, Smo		56
	Lung	Chemical	Ptch, Gli		41
	Bone	Fracture	Shh, Ptch, Smo, Gli		59, 72
Wnt pathway					
Mouse	Muscle	Ischaemia	Wnts 5a, 5b, 7a, 7b, β-catenin, Tcf	Muscle regeneration inhibited by secreted Fz peptides (SFRPs)	23
Human	Bile duct	Immune	Wnts 2, 5a, 10b, 12, 13		56
Human	Kidney	Ureteral obstruction	Wnt 4		58
Hydra	Axis	Dissociation	Tcf, Wnt, β-catenin		49
Mouse	Liver	Hepatectomy	Wnt1, β-catenin		57

Several of the organs listed also require Hh and Wnt signalling pathways during embryonic patterning. Where available, experimental evidence for pathway function in regeneration is listed under 'Pathway modulation and effect'. *A more potent derivative of cyclopamine.

The nature of the oncogenic events that may trap stem cells in an active state of renewal is not always clear. As noted above, Hh pathway activation in tissues that give rise to non-Gorlin's tumours seem not to be limited by ligand availability, but by the responsiveness to ligand. In normal prostate, the limiting factor in pathway responsiveness is SMO expression, and SMO upregulation is uniformly noted in all metastatic prostate cancer[29]. Upregulation of Smo also occurs in mice in response to injury of other endodermal tissues (P.A.B., S.S.K. and D.M.B., unpublished data), and has been dramatically demonstrated to occur locally at the site of bone injury[72]. The acquisition of pathway responsiveness through SMO upregulation is therefore a common feature of both injury response and tumorigenesis; cancer cells in this respect closely resemble cells in injured tissues. The identity and source of the signal that induces Smo expression in injured tissues may therefore lend insight into the targets of oncogenic processes that lead to SMO expression and Hh ligand responsiveness.

Tissue repair, invasion and metastasis

Most cancer deaths are caused by the growth of tumours at distant metastatic sites rather than at the site of origin. Metastasis requires the capacity to detach from the original tumour mass, to migrate through several types of tissue and to colonize a permissive ectopic site. Current evidence suggests that there may be associations between the activities of Hh and Wnt pathways and metastasis, at least in some types of cancer. In colorectal and pancreatic adenocarcinomas, Wnt and Hh pathway activities, respectively, have been linked to all stages of these diseases — from pre-invasive neoplasia to locally growing and metastatic lesions[42,43,73].

In contrast, high-level Hh pathway activity in prostate cancer is associated exclusively with metastatic tumours[29], and cell lines with and without the ability to metastasize can be interconverted by modulation of Hh pathway activity. Hh signalling specifically promotes collagen-matrix invasion and the expression of genes associated with a transition from epithelial to mesenchymal character (epithelial–mesenchymal transition, EMT). These genes include Snail, a helix-loop-helix transcriptional repressor[74]. EMT and the expression of Snail or its homologue Slug is also associated with aggressive behaviour, including metastasis, of other cancers[74,75], and has been linked to activity of the Wnt pathway in colorectal cancer[76,77].

How does the promotion of invasiveness seen in tumours relate to the physiological roles of Hh or Wnt? Migration through tissues is a normal feature of neural crest, germ cell and haematopoietic stem cell development, and is also observed during acute epithelial injury repair in adults, in a process called epithelial restitution[78]. During restitution, epithelial cells adjacent to a focally denuding injury detach from each other, assume an elongated shape and rapidly migrate (often within 2–4 hours) to the injured area, where they invade remnants of the injured tissue and reconstitute epithelial continuity. This behaviour has so far been observed in differentiated

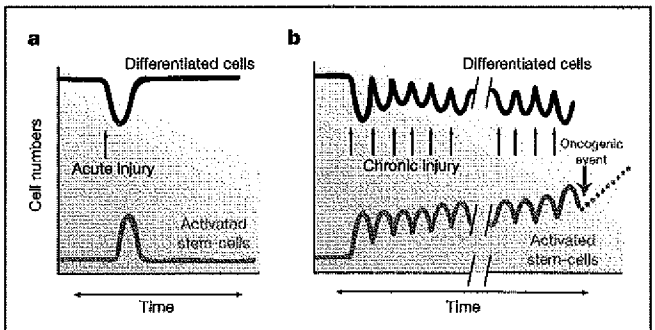

Figure 3 Increased cancer risk during chronic injury. **a**, Acute injury produces a transient expansion in the pool of activated stem cells. On completion of repair, the number of activated stem cells returns to baseline. **b**, Chronic injury also causes expansion in the pool of activated stem cells, but because of repeated injury the pool size does not return to baseline. As a result, the total number of activated stem cells is greatly increased over time, increasing the probability that an oncogenic event will trap a stem cell in the activated state.

cells, but as convenient markers for tracking stem cells in such experiments are lacking, the possibility remains that stem cells also become motile and invasive during injury repair. The acquisition of such an ability to invade and move through tissues might represent part of a programme of stem cell activation for optimal completion of epithelial repair. Hh and Wnt pathway activities are thus linked to the activation of stem cells in injury repair, and this reparative state is associated with cell behaviour that is recapitulated in metastasis. It seems plausible therefore that the trapping of stem cells in a state of repair might predispose them not only to tumour formation in general, but to the formation of aggressive tumours in particular.

Perspectives and implications

If cancer stem cells responsible for driving the growth of cancer types associated with Hh and Wnt pathway activation indeed come from stem cells trapped in a state of active renewal by pathway activities, then a logical therapeutic approach for these cancers would be to impose a state of pathway blockade (see introduction in this issue by Sawyers, page 294). Potential problems associated with such approaches might include cognitive or affective disturbances, as both Hh and Wnt pathways are active in the mature brain. In addition, the roles of pathway activities in normal stem cell renewal suggest that pathway blockade might cause a complete or partial loss of stem cell pools. Latent symptoms caused by such a loss might include an increased susceptibility to degenerative disorders, and appear only after passage of a significant fraction of lifespan. On the other hand, Hh or Wnt pathway activities might be restricted solely to the stimulation of stem cell self-renewal and not affect signals required for the maintenance of stem cell identity. In this case, endogenous stem cells may remain quiescent during pathway blockade but regain renewal capacity once therapy is completed and the blockade lifted. Stem cell niches might also persist and permit the regeneration of stem cells that are temporarily lost during a period of pathway blockade. Consistent with such a possibility, the well-characterized germline stem cell niches in the *Drosophila* ovary and testis have been reported to persist and supply continuous niche signals for a significant fraction of the adult lifespan, even after they are emptied of stem cells[13,79].

The potential success of such therapeutic approaches is suggested by the achievement of growth inhibition or regression, complete in some cases with systemic treatment by the Hh pathway antagonist cyclopamine in murine models of several Hh-pathway-associated tumour types[29,37,42,43,80]. In addition, a recent report demonstrates growth inhibition of spontaneous medulloblastomas arising in $Ptch^{+/-}p53^{-/-}$ mice on systemic treatment with a synthetic Hh pathway antagonist[81]. Cancer growth in these tumour types apparently requires an active state of renewal, without which cancer stem cells are depleted by differentiation or apoptosis. The lack of toxic effects in mice during periods of systemic cyclopamine treatment extending as long as seven weeks and during follow-up observation periods of nearly half a year also augurs well for this approach. More recently, cyclopamine-induced regression of human basal-cell carcinomas was reported[82], suggesting the potential effectiveness of Hh pathway blockade in humans. As cyclopamine application in these human cases was topical, cognitive or affective disturbances that might be caused by systemic pathway blockade cannot be ruled out. Such effects, if they materialize, might be reduced or eliminated by the development of pathway-blocking agents that do not cross the blood/brain barrier. The feasibility of such an approach is suggested by the identification of several structurally distinct classes of Hh pathway antagonists[40,83]. Some recent success has also been reported in the identification of Wnt pathway antagonists[48], suggesting that the therapeutic effects of blocking this pathway may be tested in the near future.

Systemic pathway blockade in humans may require consideration of other factors. For example, the inability of prostate epithelium or muscle to regenerate under conditions of Hh or Wnt pathway blockade may be typical of many tissues, and the loss of stem cell renewal divisions could result in increased sensitivity to injury or other transient demands being placed on stem cell pools. It may therefore be important for patients to avoid even mild sources of trauma while undergoing pathway blockade therapy. This consideration also raises doubts about combining any form of cytotoxic chemotherapy with pathway blockade, as indiscriminate injury imposed by such therapy might affect some of the tissues containing stem cells whose renewal depends on pathway activities. Despite these concerns, preliminary studies *in vitro* and in mice suggest that blockade of these pathways may offer a new and efficacious therapeutic approach to a large group of highly lethal cancers. Other strategies of potential use in cancer prevention and therapy might also arise from an improved understanding of the response to tissue injury, in particular of the signals that regulate the activation of tissue stem cells. □

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